

wherein

M is a transition metal ion;

the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-,
-N=N-, and -CH=N-;

X and X₁ are co-ligands and wherein at least one of X and X₁ is present; and,

Z is a phosphoramidite nucleotidyl moiety attached via the base.

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REMARKS

Claims 44-49 are pending. An Appendix of Pending Claims is attached for the Examiner's convenience.

Claims 44-49 are rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 44-49 are rejected under 35 U.S.C. under § 112, second paragraph for being indefinite.

Summary of The Rejections Under 35 U.S.C., §112, First and Second Paragraphs

In rejecting Claims 44-49 under 35 U.S.C. under § 112, first and second paragraphs, the Examiner's position appears to be that the specification is entirely prospective. Specifically, the Examiner argues that the specification lacks specific embodiments which show the applicant had possession of the compounds indicated, that a substantial number of compounds had been made, and that there is a complete absence of

requisite guidance to permit the ordinary practitioner to make the compounds without undue experimentation.

It is well settled law that the specification must enable the scope of the claimed invention, but that the specification need not provide a specific description for each and every embodiment covered by the claimed invention. *See, e.g., Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1115 (Fed. Cir. 1991). That the claimed invention covers a wide variety of compounds amenable to synthesis in accordance with the claimed invention is not relevant to the issue of enablement. Rather, the test of enablement requires that the specification, in light of the prior art, provides ample guidance to one of skill in the art, to make and use the compounds of the invention. Applicants submit that the specification provides specific examples of making at least two aromatic acetylene derivatives of phenanthroline. This alone is sufficient teaching to enable the scope of independent claims 44-46 and 48.

Synthesis of Aromatic Acetylene Derivatives of Phenanthrolines

The present invention is directed to the synthesis of 1,10-phenanthroline derivatives functionalized at the 3 and /or 8 positions. This is accomplished by the halogenation of 1,10-phenanthroline at the 3 and/or 8 positions. Once halogenated derivatives of 1,10-phenanthrolines are available, additional molecules can be added, such as transition metals, aromatic acetylenes, nucleic acids, nucleotides, nucleosides, proteins, etc.

The synthesis of aromatic acetylene derivatives of phenanthrolines requires:

- halogenation, particularly bromination, of 1,10-phenanthroline at the 3- and/or 8-position;
- optional addition of a transition metal to the halogenated 1,10-phenanthroline

to form transition metal derivatives of 1,10 phenanthroline; and, c) palladium cross coupling to covalently attach acetylated aromatic compounds such as nucleic acids, nucleotides and nucleosides to the halogenated 1,10 phenanthroline (which may comprise transition metals).

Halogenation of 1,10-phenanthroline begins with commercially available 1,10-phenanthroline monohydrochloride monohydrate. A typical procedure using bromine is provided in working example 1. As outlined at page 23, lines 5-25:

In a typical procedure, a solution of the 1,10-phenanthroline monohydrochloride monohydrate(10 g, 43 mmol) in nitrobenzene (20 ml) was heated to 130-140 °C in a 250 ml 3-neck flask. Bromine (3.3 l, 64 mmol in 9.3 ml nitrobenzene) was added dropwise over a period of 1 hr. Upon the addition of bromine, the 1,10-phenanthroline went into solution. After stirring for 3 hr at the same temperature, the reaction mixture was cooled to room temperature, treated with concentrated ammonium hydroxide (100 ml) and extracted with dichloromethane (3X50 ml). The combined organic layers were washed with water (3X50 ml) and dried ($MgSO_4$). Concentration in vacuum afforded a suspension of the products in nitrobenzene. The nitrobenzene was removed by dissolving the suspension in dichloromethane (10 ml) and filtering it through silica gel (300 ml) using dichloromethane as the eluent. After the nitrobenzene eluted out, the products were recovered by gradually increasing the polarity of the eluent up to 10% MeOH in CH_2Cl_2 . Flash column chromatography (0.6% MeOH in CH_2Cl_2) afforded 3-bromo-phenanthroline (3.6 g, 33% yield, m.p. 164-167°C) and the 3,8-bromo-phenanthroline (2.4 g, 17% yield, m.p. 270-273°C) as white powders. Higher solvent polarity (10% MeOH in CH_2Cl_2) elutes unreacted 1,10-phenanthroline (ca. 4 g) that can be recycled.

A typical reaction for adding transition metals to halogenated 1,10-phenanthrolines is described at page 27, lines 4-13:

In a typical reaction, the ligand **3a** (0.1 g, 0.26 mmol) in degassed DMF (10 ml) was treated under argon with a solution of K_2RuCl_4 , (33 mg, 0.08 mmol) in water (4 ml) containing 1 drop of 6N HCl. The solution was refluxed for 1 h. Sodium hypophosphite (38 mg, 0.44 mmol) in water (1 ml) was added, and reflux was continued for 1 h. After cooling to 60°C, the reaction mixture was treated with potassium hexafluorophosphate (48 mg, 0.26 mmol) as a 10% aqueous solution, cooled to RT and concentrated *in vacuo*. Silica-gel chromatography using 1% aqueous 0.5 M KNO_3 , in acetonitrile as

eluent afforded Ru(3a)₃. ¹H NMR (CD₃CN) δ 8.75 (d, *J*=1.3 Hz, 2H, H_{2,9}), 8.27 (s, 2H, H_{5,6}), 8.18 (d, *J*=1.3 Hz, 2H, H_{4,7}), 7.45 (m, 10H, phenyl).

Chemical compositions of ligand 3a and Ru(3a)₃ are found in Tables 1 and 2 of the specification respectively.

With respect to the palladium-catalyzed coupling reactions, transition metal catalyzed reactions have played a role in organic synthesis for a long time. Over twenty five years ago the use of palladium as a catalyst for coupling reactions was disclosed. Since then, the scope of palladium-catalyzed reactions has been expanded and used to join a wide variety of chemical reactants via the formation of a carbon-carbon bond. See for example, U.S. Patent No. 6,136,157; a copy of which is attached as Exhibit A, which describes the efforts devoted to extending the scope of palladium-catalyzed reactions..

Briefly, palladium-catalyzed cross coupling reactions are used to form carbon-carbon bonds between halogenated carbons and other carbon atoms, such as alkynes, alkenes and aromatic acetylenes. This reaction is quite versatile and widely used to synthesize a large number of compounds. In particular, palladium-catalyzed cross coupling reactions have been used to couple nucleosides to a variety of compounds. For example, Robins and Barr report details of a high-yield palladium-catalyzed coupling procedure which provides direct access to 5-alkynyluracil bases and nucleosides from terminal alkynes and readily available 5-iodouracil derivatives. See Robins and Barr, (1983) *J. Org. Chem.*, 48:1854-1862, a copy of which is attached as Exhibit B. Moriarty et al. describe the use of a palladium catalyzed coupling reaction between 8-ido derivatives of O-TBDMS protected adenosine, 2'-deoxyadenosine, and 2',3'-dedeoxyadenosine to obtain 8-substituted nucleosides. Moriarty, et al., (1990) *Tetrahedron Letters*, 41:5877-5880, a copy of which is attached as Exhibit C. Thus, the

use of palladium catalyzed-cross coupling reactions in the coupling of nucleosides with other compounds is known in the art.

The present invention utilizes a palladium-catalyzed cross coupling reaction to make 1,10-phenanthrolines derivatives. Guidance for palladium-catalyzed cross coupling reactions between an aromatic acetylene and halogenated 1,10-phenanthroline is depicted in Scheme II, page 18 of the specification. Other palladium-catalyzed reactions include:
a) reacting an halogenated 1,10-phenanthroline with acetylene, to form a 3- or 3,8-acetylene-phenanthroline and then coupling to an halogenated aromatic group (Scheme III, page 18 of the specification); b) coupling transition metal complexed 1,10-phenanthrolines with an aromatic acetylene (Scheme IV, page 20); c) coupling a transition metal complexed 3-acetylene phenanthroline with an aromatic bromine (Scheme V, page 20); and d) coupling an optionally transition metal complexed 1,10-phenanthroline with a halogenated nucleosides (page 20, line 8 through page 21, line 2).

Moreover, specific reaction conditions are disclosed in example 3, page 29, lines 17-24:

A representative procedure for the palladium-mediated cross-coupling reactions between **4** and aromatic acetylenes is as follows. A mixture of **4** (50 mg, 0.052 mmol), $(\text{Ph}_3\text{P})_2\text{PdCl}_2$ (4 mg, 0.0057 mmol) and CuI (0.5 mg, 0.0026 mmol) was treated with a degassed solution of 4-ethynyltoluene (11 μl , 0.11 mmol) in DMF (5 ml) and triethylamine (3 ml) for 1 hour at room temperature under Argon. The crude reaction mixture was evaporated to dryness and the product **6** was obtained in 91% yield as an orange-red powder after successive crystallizations from dichloromethane-ethanol.

The chemical structures for compounds **4** and **6** are found on pages 31 and 32 respectively.

The substrates required for the palladium cross coupling reactions described in the present invention are widely available. Aromatic acetylenes can be made using techniques well known in the art or obtained commercially. See specification at page 20, lines 3-7.

Halogenated bases and nucleosides are commercially available. See Robbins and Barr, Exhibit C. Moreover, halogenated bases and nucleosides can be synthesized using terminal alkynes and halogenated nucleosides as starting materials. See Moriarty et al., Exhibit B.

In further support of the enablement of the specification, applicants draw the Examiner's attention to a recent article which describes the synthesis of metal containing nucleosides. See Hurley and Tor (1998), *J. Am. Chem. Soc.*, 120:2194-2195, enclosed herein as Exhibit D. The applicants are not using subsequent work to supplement the disclosure of the application; rather, the subsequent work is presented to show that the utility asserted and shown in the application is supported by further research, and that the specification fully enables the synthesis of 1,10-phenanthroline derivatives substituted at the 3- and 8-positions. See In re Wilson, 135 USPQ 442, 444 (CCPA 1962); Ex parte Obukowicz, 27 USPQ 2d 1063 (BPAI 1993); Gould v. Quigg, 3 USPQ 2d 1302, 1305 (Fed. Cir. 1987):

"it is true that a later dated publication cannot supplement an insufficient disclosure in a prior dated application to render it enabling. In this case the later dated publication was not offered as evidence for this purpose. Rather, it was offered . . . as evidence that the disclosed device would have been operative."

Hurley and Tor report the synthesis of novel Ru^{II}- and Os^{II}-containing nucleosides and their phosphoramidite derivatives. The methods used to synthesize these metal-modified nucleosides are the same as those disclosed in the present invention and outlined above: a) halogenation of 1,10-phenanthrolines; b) the optional incorporation of transition metals to form transition metal complexed 1,10-phenanthroline derivatives, and, c) palladium-catalyzed cross coupling reactions between acetylated nucleosides and transition metal complexed 1,10-phenanthrolines. See Exhibit D, page 2194. In addition,

the Hurley and Tor reference utilize solid phase phosphoramidite chemistry to synthesize the corresponding metal-modified phosphoramidite. Exhibit D, page 2194; the specification, page 21, lines 1-2.

In light of the prior art and teachings in the specification, applicants submit that the skilled artisan would find guidance for making the compounds described in the specification. Accordingly, applicants submit that Claims 44-49 are enabled and request withdrawal of the rejections under 35 U.S.C. § 112, first and second paragraphs.

The Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 44-46 and 48 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Examiner indicates the subject matter of the instant claims is indefinite because the attachment point of the “Z” substituent has not been defined.

Applicants have amended claims 44-46 and 48 to clarify the attachment point of “Z” is via base of the nucleoside. Support for the base as the attachment point is found in the specification at page 11, line 23 through page 13, line 12. Accordingly, applicants respectfully request withdrawal of the rejection.

The Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 44-49 are rejected under 35 U.S.C. § 112, first paragraph because the term “M is a transition metal ion” lacks enabling support. As stated above, support for the synthesis of transition metal complexed derivatives of 1,10-phenanthroline is found in the specification at page 27, lines 4-13. The specification, at page 7, lines 13-18 provides a list of metals which can be used in the invention:

... M is a metal atom, with transition metals being preferred. Suitable transition metals for use in the invention include, but are not limited to, Cadmium (Cd), Copper (Cu), Cobalt (Co), Zinc (Zn), Iron (Fe), Ruthenium (Ru), Rhodium (Rh), Osmium (Os) and Rhenium (Re), with Ruthenium, Rhenium and Osmium being preferred and Ruthenium(II) being particularly preferred.

Further support for the synthesis of transition metal complexed 1,10-phenanthroline is found in Exhibit D, which discloses the synthesis of (bpy)₂Ru(3-bromo-1,10-phenanthroline)²⁺ and (bpy)₂Os(3-bromo-1,10-phenanthroline)²⁺. Accordingly, Applicants request withdrawal of the rejection.

The Applicants submit that the claims are now in condition for allowance and an early notification of such is respectfully solicited.

The Commissioner is authorized to charge any additional fees, including any extension fees, which may be required, or credit any overpayment to Deposit Account No. 06-1300 (order No. A63463-1/RFT/RMS).

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Robin M. Silva

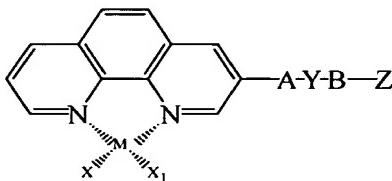
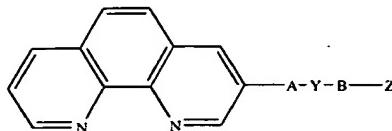
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APPENDIX OF PENDING CLAIMS

44. (Twice Amended) A compound represented by one of the formulae:



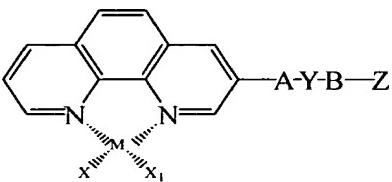
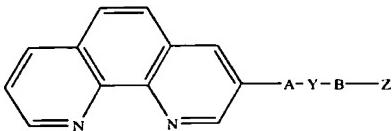
wherein

M is a transition metal ion;

the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;

X and X₁ are co-ligands and wherein at least one of X and X₁ is present; and Z is a nucleosidyl moiety attached via the base.

45. (Twice Amended) A compound represented by one of the formulae:



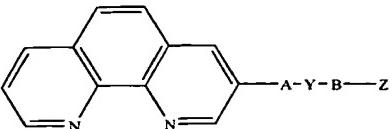
wherein

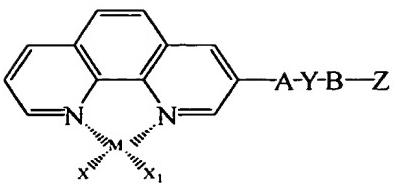
M is a transition metal ion;

the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;

X and X₁ are co-ligands and wherein at least one of X and X₁ is present; and Z is a nucleotidyl moiety attached via the base.

46. (Twice Amended) A compound represented by one of the formulae:





wherein

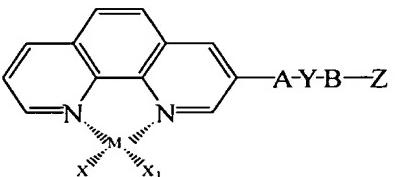
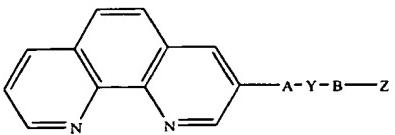
M is a transition metal ion;

the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;

X and X₁ are co-ligands and wherein at least one of X and X₁ is present; and,
Z is a nucleic acid moiety attached via a base.

47. A compound according to claim 46, wherein said nucleic acid moiety comprises a nucleic acid analog.

48. (Twice Amended) A compound represented by one of the formulae:



wherein

M is a transition metal ion;

the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;

X and X₁ are co-ligands and wherein at least one of X and X₁ is present; and,
Z is a phosphoramidite nucleotidyl moiety attached via the base.

49. A compound according to claims 44, 45, 46 or 48 wherein M is selected from the group consisting of ruthenium, rhenium and osmium.